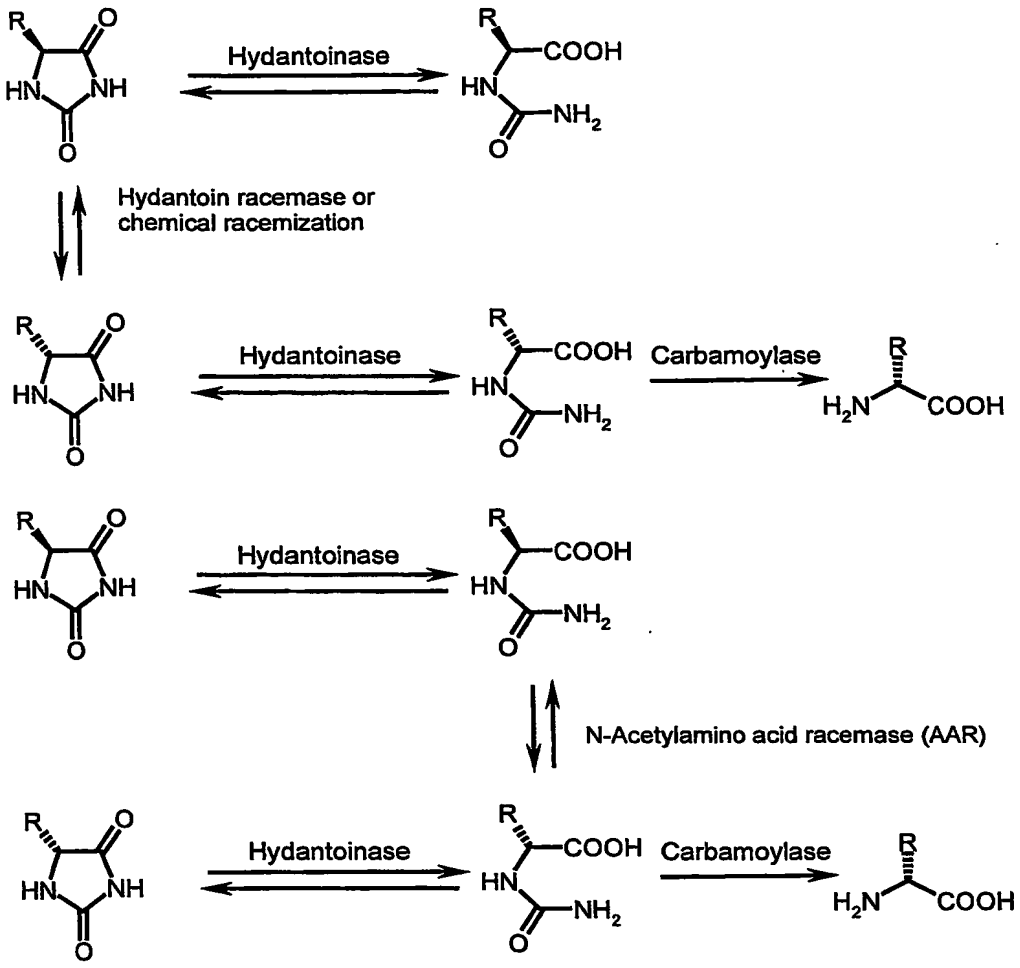
Mutants for the preparation of D-amino acids

The present invention relates to a process for the preparation of D-amino acids. In particular, these are obtained enzymatically via the so-called hydantoinase route using recombinant microorganisms. The present invention likewise relates to microorganisms modified in this way.

D-Amino acids are compounds which are often employed in organic synthesis as intermediates for the preparation of pharmaceutical active compounds.

Enzymatic hydrolysis of 5-substituted hydantoins to give N-carbamoyl-amino acids and further reaction thereof to give the corresponding enantiomerically enriched amino acids is a standard method in organic chemistry ("Enzyme Catalysis in Organic Synthesis", eds.: Drauz, Waldmann, VCH, 1st and 2nd ed.). The enantiodifferentiation can take place here either at the stage of hydantoin hydrolysis by hydantoinases, or optionally during cleavage of N-carbamoylamino acids by means of enantioselective carbamoylases. Since the enzymes in each case convert only one optical antipode of the corresponding compound, attempts are made to racemize the other in the mixture (in situ) in order to ensure complete conversion of the racemic hydantoin, which is easy to prepare, into the corresponding enantiomerically enriched amino acid. The racemization can take place here either at the stage of the hydantoins by means of chemical (base, acid, elevated temp.) or enzymatic processes, or can proceed at the stage of the N-carbamoylamino acids by means of e.g. acetylamino acid racemases (DE10050124). The latter variant of course functions successfully only if enantioselective carbamoylases are employed. The following equation illustrates this state of affairs.

Equation 1:



It has been found that the use of recombinant microorganisms which have hydantoinase, carbamoylase and racemase activities for the preparation of various D-amino acids presents problems. Fig. 1 shows the conversion of hydroxymethylhydantoin and ethylhydantoin with *E.coli* JM109 transformed with a D-carbamoylase and D-hydantoinase from *Arthrobacter crystallopoietes* DSM 20117 (in accordance with the patent application DE10114999.9 and DE10130169.3). The reaction conditions are chosen according to example 1.

As fig. 1 shows by way of example, in the conversion of various 5-monosubstituted hydantoins, marked breakdown of the D-amino acids formed takes place. This reduces the yield which can be achieved and makes working up of the product difficult.

The expert knows that various enzymes, such as D-amino acid oxidases [EC 1.4.3.3], D-amino acid dehydrogenases [EC 1.4.99.1], D-amino acid aminotransferases [EC 2.6.1.21], D-amino acid N-acetyltransferases [EC 2.3.1.36], D-hydroxyamino acid dehydratases [EC 4.2.1.14] and D-amino acid racemases [EC 5.1.1.10] can participate in the breakdown of D-amino acids. Various methods for inactivating these genes in a targeted or also non-targeted manner are also known to the expert [The pKNOCK series of broad-host-range mobilizable suicide vectors for gene knockout and targeted DNA insertion into the chromosome of Gram-negative bacteria. Alexeyev, Mikhail F. BioTechniques (1999), 26(5), 824-828; One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products, Datsenko, Kirill A. and Wanner, Barry L. PNAS (2000), 97(12), 6640-6645; D-amino acid dehydrogenase of Escherichia coli K12: positive selection of mutants defective in enzyme activity and localization of the structural gene, Wild, Jadwiga and Klopotowski, T. Mol.Gen.Genet. (1981), 181(3), 373-378.].

Unfortunately, however, the effect to be expected on cell growth when the various enzymes are inactivated is unknown and unforeseeable. What enzyme or whether a combination of various enzymes has to be inactivated in order to reduce the breakdown of a particular D-amino acid to the desired extent also cannot be predicted.

The object of the present invention was therefore to provide a microorganism which is capable of production of D-amino acids via the carbamoylase/hydantoinase route and helps to render possible a higher yield of D-amino acid

produced. It should be possible to employ this advantageously on an industrial scale under economic and ecological aspects. In particular, it should have very good growth properties under the usual economically appropriate conditions, and a sufficient genetic and physical stability and a sufficiently fast rate of conversion for hydantoins.

This object is achieved according to the claims. Claims 1 to 5 relate to particular microorganisms modified in this way, while claims 6 and 7 protect a process for the preparation of D-amino acids.

By providing a recombinant microorganism for the preparation of D-amino acids starting from N-carbamoylamino acids or 5-monosubstituted hydantoins in which the gene which codes for a D-amino acid oxidase and/or the gene which codes for a D-serine dehydratase is inactivated by mutagenesis, the objects mentioned are surprisingly and nevertheless advantageously achieved. In particular, it is to be considered surprising that microorganisms with the gene profile according to the invention which have been produced by a recombinant method are in fact stable and are capable of producing D-amino acids to an extent sufficient for industrial orders of size.

Microorganisms for recombinant embodiments which can be used are in principle all the organisms possible to the expert for this purpose, such as fungi, e.g. *Aspergillus sp.*, *Streptomyces sp.*, *Hansenula polymorpha*, *Pichia pastoris* and *Saccharomyces cerevisiae*, or also prokaryotes, such as *E. coli* and *Bacillus sp.* Microorganisms of the genus *Escherichia coli* can be regarded as preferred microorganisms according to the invention. The following are very particularly preferred: *E. coli* XL1 Blue, NM 522, JM101, JM109, JM105, BL21, W3110, RR1, DH5α, TOP 10^- or HB101. Organisms modified in

this way can be produced by methods familiar to the expert. This serves to multiply and produce a sufficient amount of the recombinant enzymes. The processes for this are well-known to the expert (Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989), Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York).

The said nucleic acid sequences are thus cloned into a host organism with plasmids or vectors by known methods and the polypeptides expressed in this way can be detected with suitable screening methods. All the possible detection reactions for the molecules formed are in principle suitable for the detection. In particular, detection reactions which are suitable in principle are all the possible detection reactions for ammonia and ammonium ions, such as Nessler reagent (Vogel, A., I., (1989) Vogel's textbook of quantitative chemical analysis, John Wiley & Sons, Inc., 5th ed., 679-698, New York), the indophenol reaction, also called Berthelot's reaction (Wagner, R., (1969) Neue Aspekte zur Stickstoffanalytik in der Wasserchemie, Vom Wasser, VCH-Verlag, vol. 36, 263-318, Weinheim), in particular enzymatic determination by means of glutamate dehydrogenase (Bergmeyer, H.,U., and Beutler, H.-O. (1985) Ammonia, in: Methods of Enzymatic Analysis, VCH-Verlag, 3rd edition, vol. 8: 454-461, Weinheim) and also detection with ammonium-sensitive electrodes. HPLC methods are furthermore used for detection of amino acids, such as e.g. a derivative method based on o-pthaldialdehyde and N-isobutyryl-cysteine for enantiomer separation of amino acids (Brückner, H., Wittner R., and Godel H., (1991), Fully automated high-performance liquid chromatographic separation of DL-amino acids derivatized with o-Phthaldialdehyde together with N-isopropyl-cysteine. Application to food samples, Anal. Biochem. 144, 204-206).

Possible plasmids or vectors are in principle all the embodiments available to the expert for this purpose. Such

plasmids and vectors can be found e.g. in Studier and colleagues (Studier, W. F.; Rosenberg A. H.; Dunn J. J.; Dubendroff J. W.; (1990), Use of the T7 RNA polymerase to direct expression of cloned genes, Methods Enzymol. 185, 61-89) or the brochures of Novagen, Promega, New England Biolabs, Clontech or Gibco BRL. Further preferred plasmids and vectors can be found in: Glover, D. M. (1985), DNA cloning: A Practical Approach, vol. I-III, IRL Press Ltd., Oxford; Rodriguez, R.L. and Denhardt, D. T (eds) (1988), Vectors: a survey of molecular cloning vectors and their uses, 179-204, Butterworth, Stoneham; Goeddel, D. V. (1990), Systems for heterologous gene expression, Methods Enzymol. 185, 3-7; Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989), Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York.

Particularly preferred cloning vectors of D-carbamoylases in *E.coli* are, for example, derivatives of pBR322, pACYC184, pUC18 or pSC101, which can carry constitutive and also inducible promoters for expression control. Particularly preferred promoters are lac, tac, trp, trc, T3, T5, T7, rhaBAD, araBAD, λpL and phoA promoters, which are sufficiently known to the expert [Strategies for achieving high-level expression of genes in *Escherichia coli*, Makrides S.C. Microbiol.Rev. 60(3), 512-538].

The inactivation of the D-amino acid oxidase (dadA) or D-serine dehydratase (dsdA) of these organisms is carried out here by methods described above, which are known to the expert. For production of the recombinant embodiments of the D-serine dehydratase- or D-amino acid oxidase-deficient strains with D-carbamoylase activity, the fundamental molecular biology methods are thus known to the expert (Sambrook, J.; Fritsch, E. F. and Maniatis, T. (1989), Molecular cloning: a laboratory manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York). Gene sequences of various D-carbamoylases e.g. from *Agrobacterium sp.*, *Arthrobacter sp.* or *Bacillus sp.* and

Ralstonia pickettii, which are preferably used, are likewise known (inter alia from US 5858759, US 5807710, US 6083752, US 6083752, US 6083752, US 6083752, US 6083752). The same methods can be used for the production of organisms which additionally contain a hydantoinase and optionally a hydantoin or carbamoyl racemase. Preferred hydantoinases which are to be employed here are those from *Thermus sp.*, *Bacillus sp.*, *Mycobacterium sp.*, *Corynebacterium sp.*, *Agrobacterium sp.*, *E.coli*, *Burkholderia sp.*, *Pseudomonas sp.*, or *Arthrobacter sp.* Hydantoin racemase can preferably be used from *Pseudomonas* sp., *Arthrobacter* sp., or *Agrobacterium* sp., optionally with the addition of auxiliary substances, such as metal ions, for example Mn^{2+} ions.

It was thus possible to produce the successful mutants Escherichia coli DSM 15181 and Escherichia coli DSM 15182. These therefore form, together with the further mutants which can be derived from them, the next subject matter of the present invention.

In the process which is likewise according to the invention, e.g. a hydantoin is converted with the said cells or cell constituents in a suitable solvent, such as, for example, water, to which further water-soluble or water-insoluble organic solvents can be added, at pH values of between 6.0 and 11, preferably between 7 and 10, and a temperature of between 10 °C and 100 °C, preferably between 30 °C and 70 °C, particularly preferably between 37 °C and 60 °C. The enzymes in question can also be used in the free form for the use. The enzymes can furthermore also be employed as a constituent of an intact guest organism or in combination with the broken-down cell mass of the host organism, which has been purified to any desired extent.

It is also possible to use the recombinant cells in flocculated, cross-linked or immobilized form, for example using agar, agarose, carrageenan, alginates, pectins,

chitosan, polyacrylamides and other synthetic carriers (Chemical aspects of immobilized systems in biotechnologies. Navratil, Marian; Sturdik, Ernest. Chemicke Listy (2000), 94(6), 380-388; Industrial applications of immobilized biocatalysts and biomaterials. Chibata, Ichiro. Advances in Molecular and Cell Biology (1996), 15A(Biochemical Technology), 151-160; Immobilization of genetically engineered cells: a new strategy for higher stability. Kumar, P. K. R.; Schuegerl, K. Journal of Biotechnology (1990), 14(3-4), 255-72.).

A process for the preparation of D-amino acids with a microorganism according to the invention accordingly forms the next subject matter of the invention. D-Aminobutyric acid, D-serine, D-methionine, D-tryptophan and D-phenylalanine are preferably prepared.
Organisms with D-carbamoylase-active and hydantoinase-active and dadA-inactivated and/or dsdA-inactivated cells are preferably used in this process for the preparation of D-amino acids. It should be mentioned here that both L-, D- or DL-carbamoylamino acids and 5-monosubstituted hydantoins, which can be converted into the corresponding carbamoylamino acids via sufficiently known hydantoinases, are possible as the educt ("Enzyme Catalysis in Organic Synthesis", eds.: Drauz, Waldmann, VCH, 1st and 2nd ed.). The dadA- and/or dsdA-deficient strains used can co-express here the carbamoylase and hydantoinase, optionally also a hydantoin racemase or carbamoylamino acid racemase, and can be employed either in the free or in the immobilized form (see above).

As has now been found, the inactivation of various enzymes is necessary in order to reduce the breakdown to a sufficient extent (< 10% breakdown within > 10 hours) for various D-amino acids (see fig. 2). For the breakdown of D-serine it has been found, surprisingly, that the inactivation of the gene of the D-amino acid oxidase (*dadA*) is not sufficient to reduce breakdown thereof

effectively. For an effective reduction in the breakdown of this amino acid, D-serine hydratase had to be additionally inactivated. In contrast to this, it had been reported in the literature that a breakdown of D-serine reduced > 3-fold is achieved by an inactivation of dadA [D-Amino acid dehydrogenase of Escherichia coli K12: positive selection of mutants defective in enzyme activity and localization of the structural gene. Wild, J.; Klopotowski, T. Mol. Gen. Genet. (1981), 181(3), 373-378]. Likewise in contrast to the results described there, it has been found, surprisingly, that D-serine is broken down very much faster than, for example, D-methionine.

In contrast to D-serine, the breakdown of aromatic and aliphatic D-amino acids, such as, for example, D-phenylalanine, D-methionine or D-aminobutyric acid, is achieved sufficiently by an inactivation of the D-amino acid oxidase. However, for D-phenylalanine, surprisingly, both deletions (ΔdsdA & ΔdadA) show a positive effect, while for D-methionine the deletion in dsdA shows no additional effect. These results are summarized in fig. 2 (Breakdown of various amino acids with various mutants of *E.coli* BW25113. *E.coli* ET3 has a deletion of the D-amino acid oxidase (ΔdadA); *E.coli* ET4 additionally has a deletion of D-serine dehydratase (ΔdsdA). For the reaction conditions see example 3).

The literature references cited in this specification are regarded as also included in the disclosure.

The organisms DSM15181 (ET3) and DSM15182 (ET4) were deposited by Degussa AG on 04.09.2002 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen [German Collection of Microorganisms and Cell Cultures], Mascheroder Weg 1b, D-38124 Braunschweig.

Examples

Example 1: Production of D-amino acids by means of recombinant *E.coli* cells

Chemically competent *E.coli* JM109 (Promega) were transformed with pJAVI16 (see fig. 3). This plasmid carries a D-carbamoylase and a D-hydantoinase from *Arthrobacter crystallopoietes* DSM20117. The sequences of the D-hydantoinase and D-carbamoylase are shown in Seq. 1 and 3 (see also DE10114999.9 and DE10130169.3).

The *E.coli* cells transformed with pJAVIER16 were placed individually on LBamp plates (ampicillin concentration: 100 µg/ml). 2.5 ml LBamp medium with 1 mM $ZnCl_2$ were inoculated with an individual colony and incubated for 30 hours at 37 °C and 250 rpm. This culture was diluted 1:50 in 100 ml LBamp medium with 1 mM $ZnCl_2$ and 2 g/l rhamnose and incubated for 18 h at 30 °C. The culture was centrifuged for 10 min at 10,000 g, the supernatant was discarded and the biomass was weighed. Various hydantoin derivatives, e.g. 100 mM DL-hydroxymethylhydantoin or DL-ethylhydantoin, pH 7.5, were added to the biomass so that a biomass concentration of 40 g moist biomass per litre results. The reaction solution was incubated at 37 °C. After various periods of time, samples were taken and centrifuged and the amino acids formed were quantified by means of HPLC.

Example 2: Production of DsdA- and DadA-deficient *E.coli* strains

DadA was deleted in *E.coli* BW25113 (deposited at CGSC under number CGSC7636) by the method described by Datsenko & Wanner (One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products, Datsenko, Kirill

A. and Wanner, Barry L. PNAS (2000), 97(12), 6640-6645). The following primers were used for this for amplification of the chloramphenicol resistance from pKD13 (deposited at CGSC under number CGSC7633):

5'_AACCAGTGCCGCGAATGCCGGGCAAATCTCCCCCGGATATGCTGCACCGTATTCCG GGGATCCGTCGACC_3' : Seq. 5

5'_AGGGGTACCGGTAGGCGCGTGGCGCGGATAACCGTCGGCGATTCCGGGG

ATCCGTCGACC-3' : Seq. 6

A transformation of the amplified product in *E.coli* BW25113 (pKD46) (deposited at CGSC under number CGSC7630) and selection of kanamycin-resistant clones rendered possible the isolation of *E.coli* ET2. After removal of the chloramphenicol resistance in accordance with the protocol of Datsenko & Wanner, it was possible to isolate the strain *E.coli* ET3. For the deletion of dsdA in *E.coli* ET3, the chloramphenicol resistance from pKD13 was in turn amplified with the following primers:

5'_GCGGGCACATTCCTGCTGTCATTTATCATCTAAGCGCAAAGAGACGTACTGTGTAG GCTGGAGCTGCTTC_3' : Seq. 7

5'_GCAGCATCGCTCACCCAGGGAAAGGATTGCGATGCTGCGTTGAAACGTTAATGGGA ATTAGCCATGGTCC_3' : Seq. 8

Transformation of the amplified product in *E.coli* ET3 (pKD46) and selection of kanamycin-resistant clones rendered possible the isolation of *E.coli* ET4, which carries a deletion both in dadA and in dsdA.

Example 3 Investigation of the breakdown of D-amino acids

2.5 ml LB medium were inoculated with an individual colony of *E.coli* BW25113, *E.coli* ET3 and *E.coli* ET4 and incubated for 18 hours at 37 °C and 250 rpm. These cultures were

diluted 1:50 in 100 ml LB medium and incubated for 18 h at 37 °C. The cultures were centrifuged for 10 min at 10,000 g, the supernatant was discarded and the biomass was weighed. Various 100 mM D-amino acid solutions, pH 7.5 (e.g. D-methionine, D-phenylalanine, D-aminobutyric acid, D-serine) were added to the biomass so that a biomass concentration of 100 g moist biomass per litre results. These reaction solutions were incubated at 37 °C and centrifuged after 10 hours. The clear supernatant was analysed for the remaining amino acid concentration by means of HPLC. The %breakdown stated was calculated from the quotient of the starting concentration and the final concentration after incubation for 10 hours.

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Degussa AG
Projekthaus Biotechnologie
Rodenbacher Chaussee 4
D-63457 Hanau-Wolfgang

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: ET3	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 15181

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION.

The microorganism identified under I. above was accompanied by:

( ) a scientific description
(X) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2002-09-04 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiss Date: 2002-09-10

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 12/2001

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

Degussa AG
Projekthaus Biotechnologie
Rodenbacher Chaussee 4
D-63457 Hanau-Wolfgang

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Degussa AG Address: Projekthaus Biotechnologie Rodenbacher Chaussee 4 D-63457 Hanau-Wolfgang	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 15181 Date of the deposit or the transfer[1]: 2002-09-04

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on[2] 2002-09-04.
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): U. Weiß Date: 2002-09-10

[1] Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
[2] In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
[3] Mark with a cross the applicable box.
[4] Fill in if the information has been requested and if the results of the test were negative.

Form DSMZ-BP/9 (sole page) 12/2001

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

Degussa AG
Projekthaus Biotechnologie
Rodenbacher Chaussee 4
D-63457 Hanau-Wolfgang

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
ET4	DSM 15182

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
(x) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2002-09-04 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name:	DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH
Address:	Mascheroder Weg 1b D-38124 Braunschweig

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):

U. Wirth

Date: 2002-09-10

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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PCT/EP03/11432

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VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Degussa AG Address: Projekthaus Biotechnologie Rodenbacher Chaussee 4 D-63457 Hanau-Wolfgang	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 15182 Date of the deposit or the transfer[1]: 2002-09-04

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2002-09-04[2]
On that date, the said microorganism was

(X)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weiss Date: 2002-09-10

1 Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
2 In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
3 Mark with a cross the applicable box.
4 Fill in if the information has been requested and if the results of the test were negative.

Form DSMZ-BP/9 (sole page) 12/2001